

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

App. No. : 10/699,512 Confirmation No. 3570
Applicant : Bennett, G.N.
Filed : October 31, 2003
TC/A.U. : 1637
Examiner : Fredman, J.N.
Docket No. : 31175413-003002
Customer No. : 51738
Entitled : RECOMBINATION ASSEMBLY OF LARGE DNA FRAGMENTS

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GEORGE N. BENNETT UNDER 37 CFR § 1.131

I, George N. Bennett, Declare as follows:

I am at least 18 years of age and am competent in all respects to make the following statements.

1. I am the sole inventor for claims 1-8 currently pending in US Patent Application No. 10/699,512.
2. The work presented in US Patent Application No. 10/699,512 was conceived prior to December 10, 1999.
3. Although the dates have been redacted, the attached laboratory PowerPoint presentation (Exhibit A) demonstrates the conception or practice of the invention prior to December 10, 1999.
4. Although the dates have been redacted, the attached laboratory notebook (Exhibit B) demonstrates the conception or practice of the invention prior to December 10, 1999.

I further declare that all statements made herein of my own knowledge are true and made on information believed to be true; further that these statements were made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of any application for which it is used.

Dated: Dec 5 2006

Respectfully submitted,

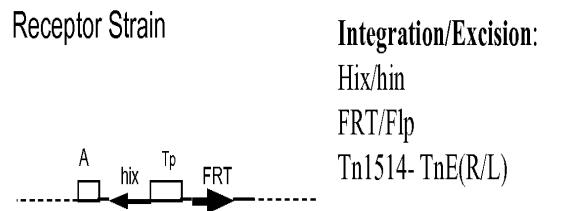
By 

Dr. George N. Bennett, Ph.D.
Department Chair
Dept. of Biochemistry and Cell Biology
Rice University
Houston, TX

EXHIBIT A

Chromosomal integration of large designer DNA into *E. Coli*

Figure 1. Components for DNA Integration



Vector A

Counter-selection:
rpsL streptomycin
sensitivity
SacB sucrose sensitivity

Antibiotic Selection:
Km kanamycin resistance
Tp trimethopin resistance
Gm gentamycin resistance

Plasmid loss selection:
ori ts temperature sensitive
replicon

Vector B

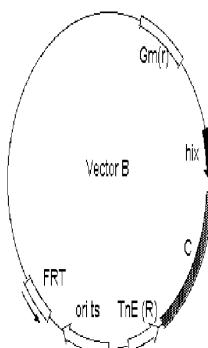


Figure 2. Integration and Excision Scheme

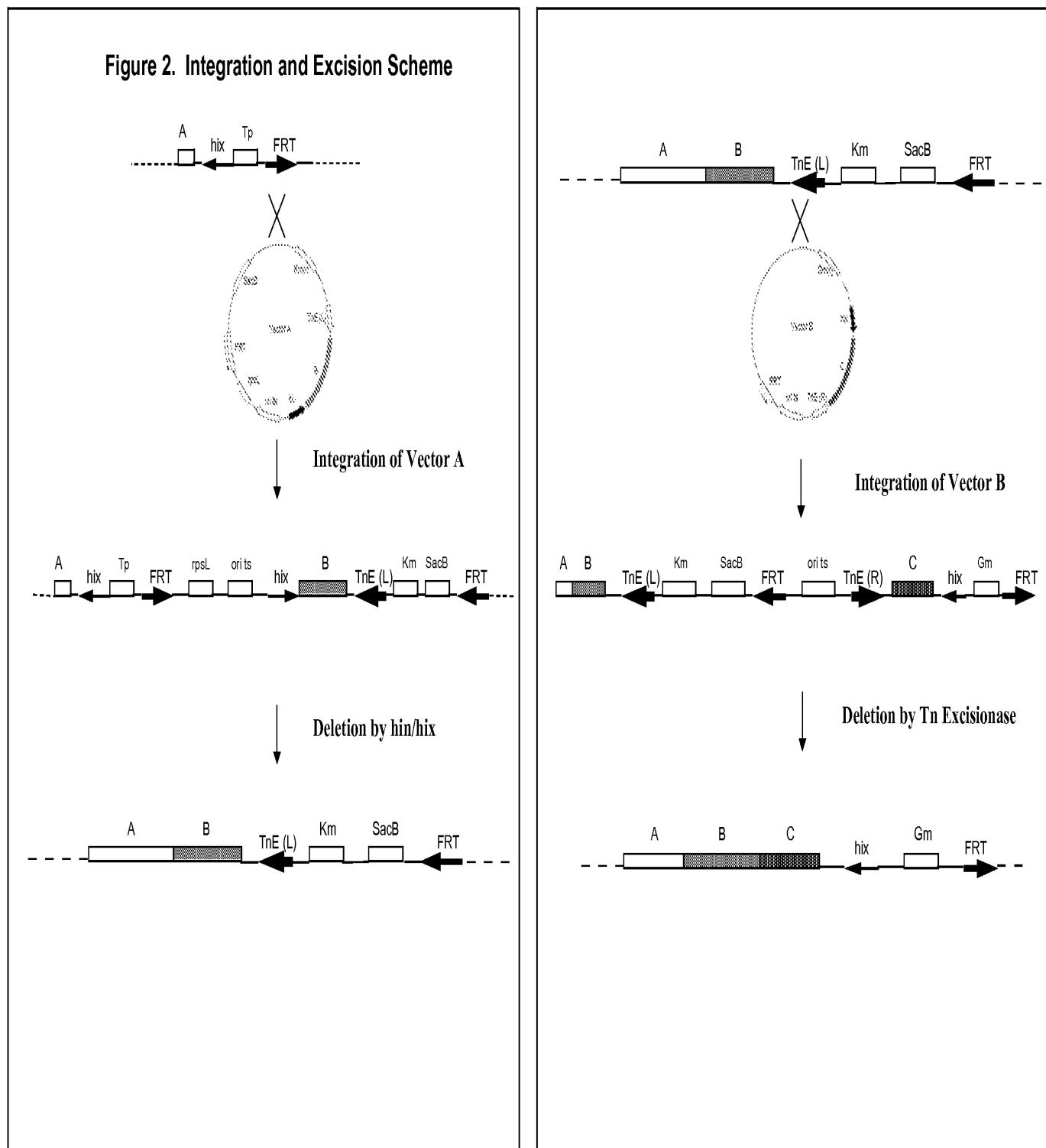


Figure 3. Construction of Receptor Strain

Susy McKay

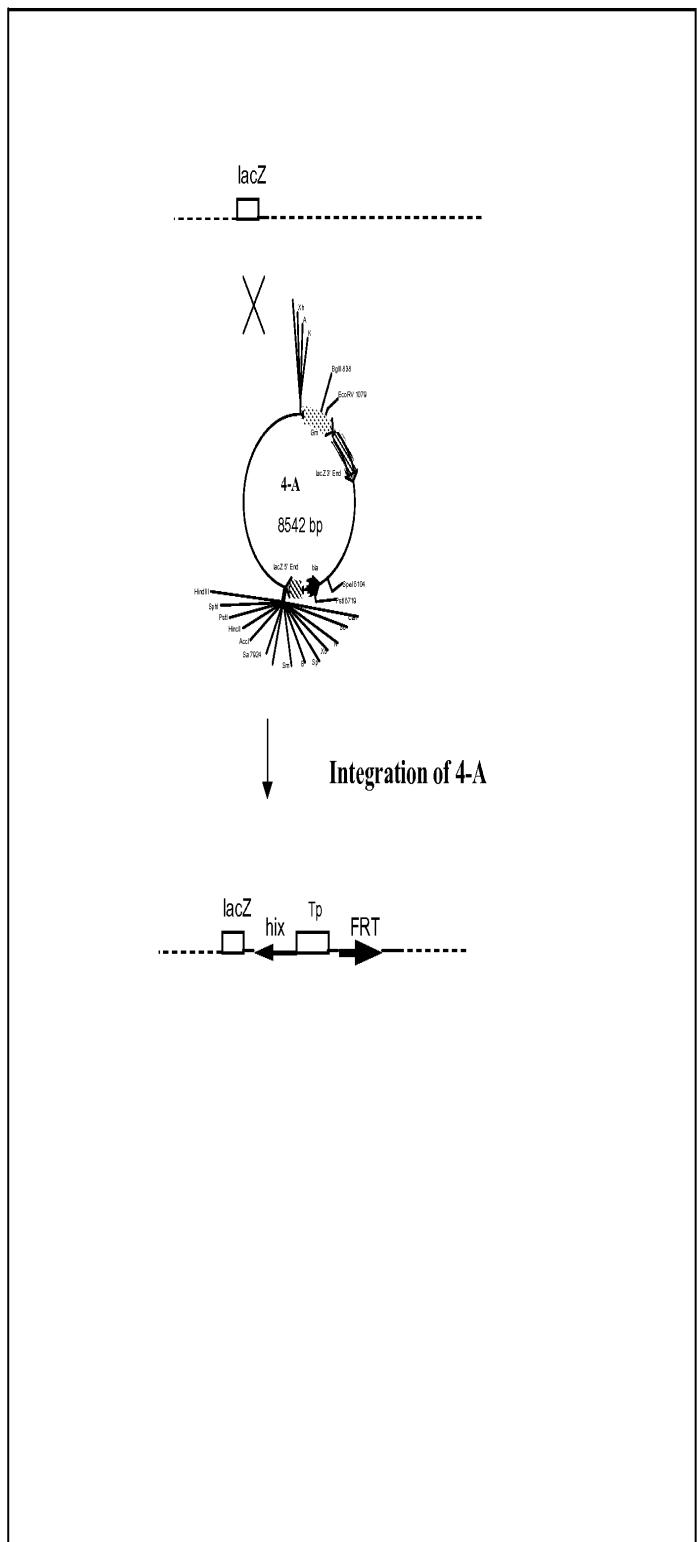
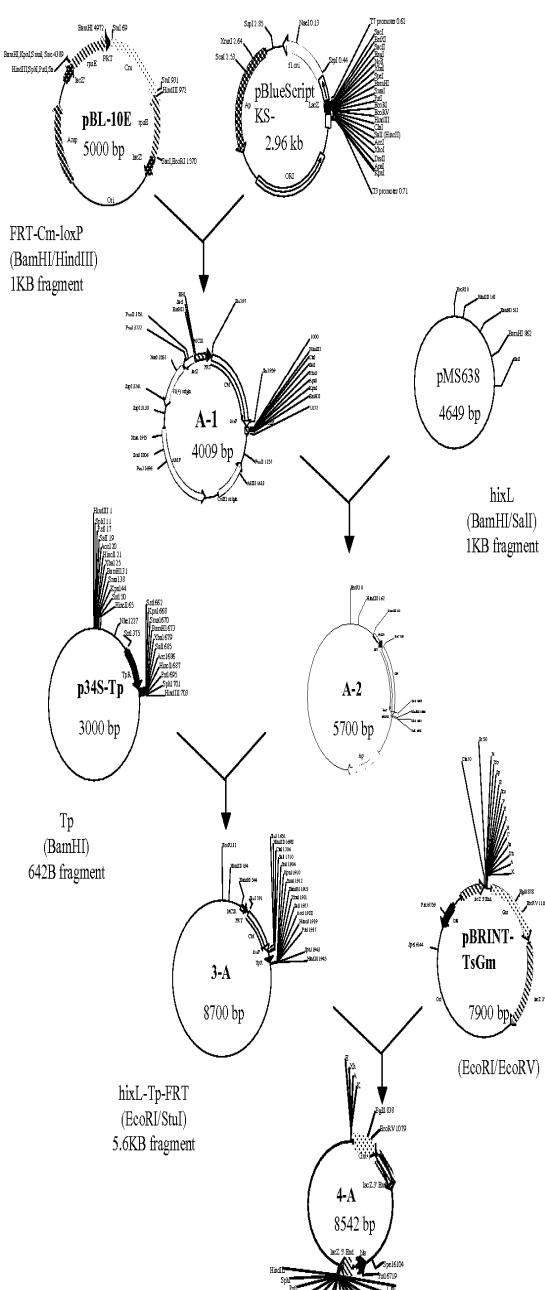


Figure 4. Construction of Vector A

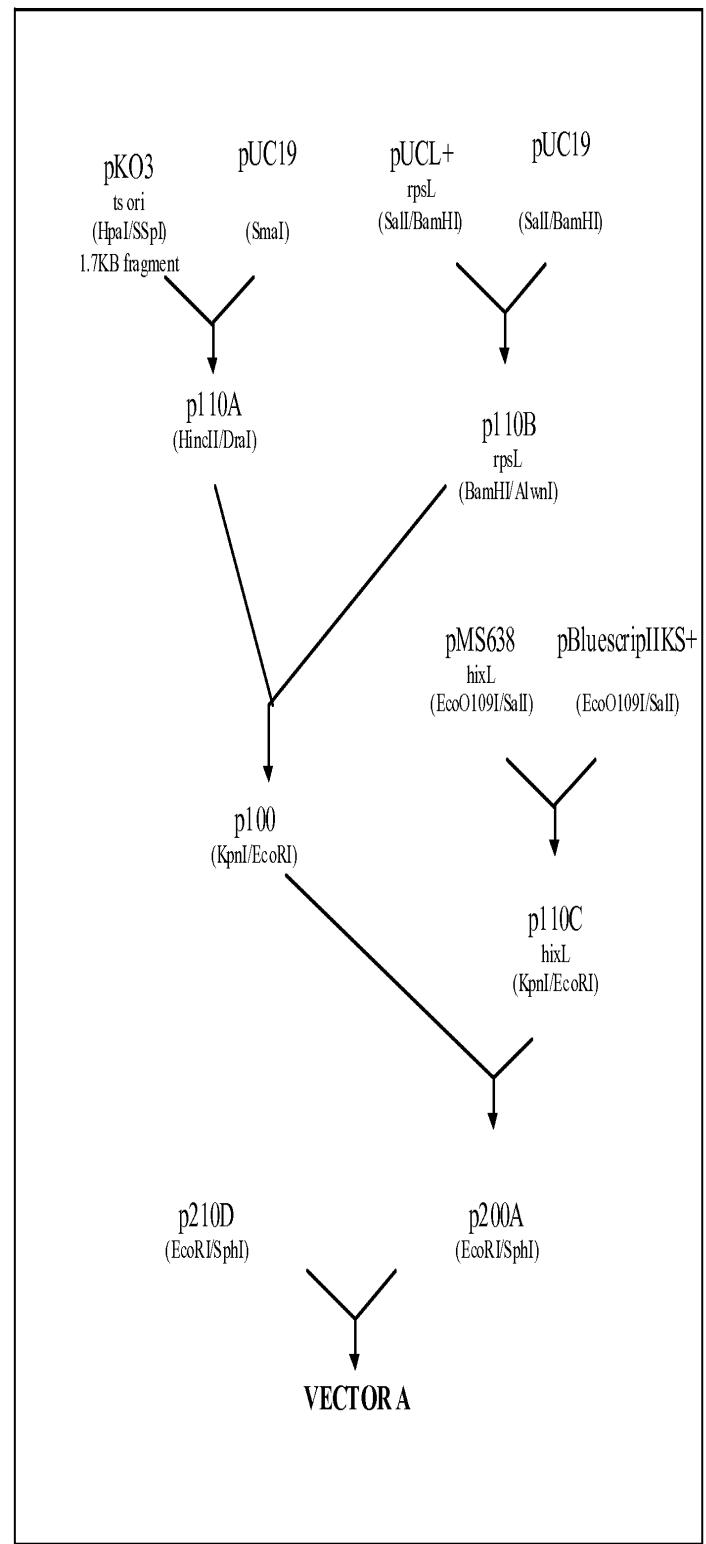
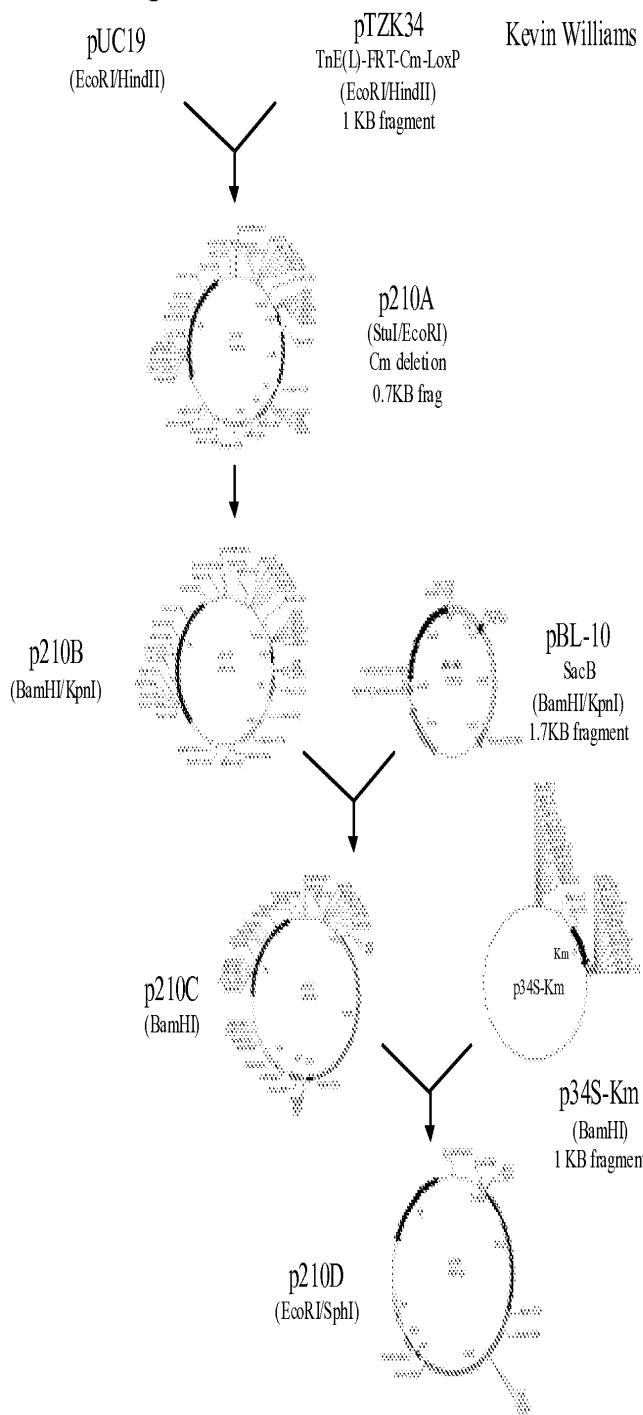


Figure 5. Construction of Vector B

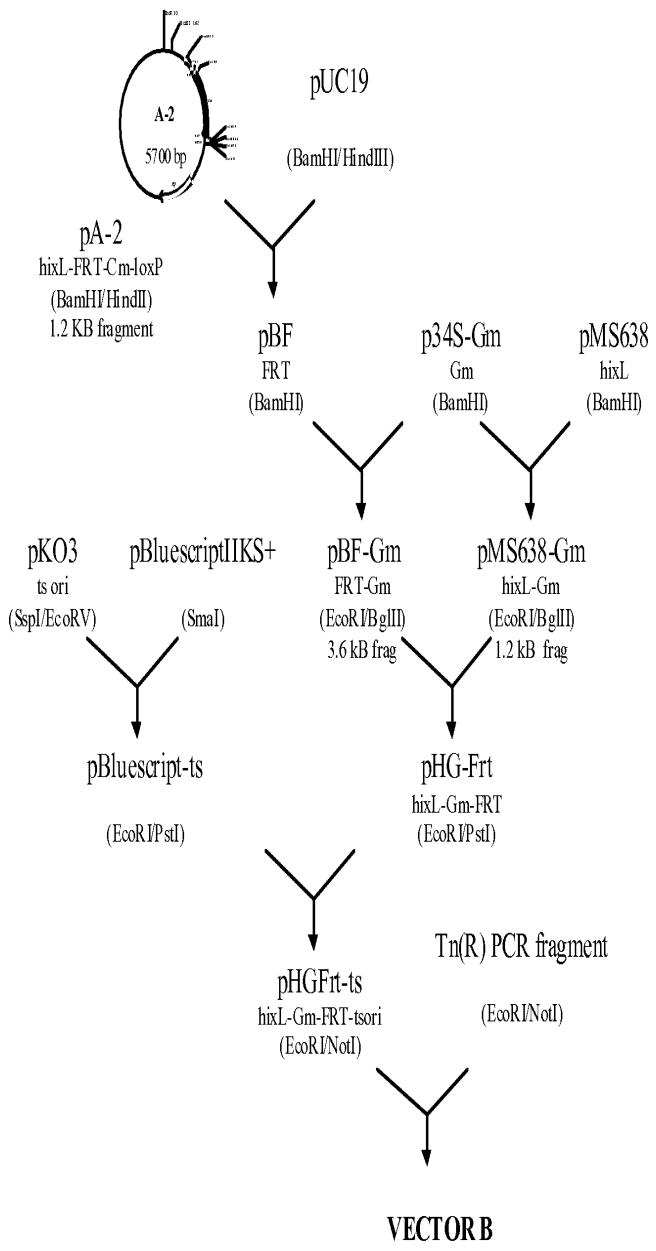
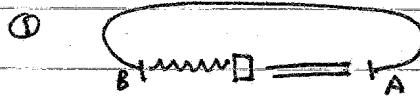


EXHIBIT B

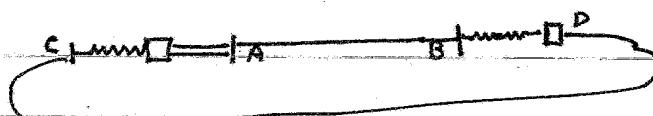


region AB cloned into plasmid ①
specif recomb site II (eg fLP yeast)

~ = conjugative transposon seq, (eg Tn916)
selection markers,
replication functions as desired



② recipient plasmid or chromosome DNA
bearing 1/2 transposon seq. and □



double X marks at II
made by selection for marker =



removal of cons transposon
precise excision (eg Tn916)
+ selection by loss of
gene at =

reiterate with successive
removals ①

joining of fragments AB, CD

at specific junction without depending
on sequence at end or within segments

control of FLP or transposon excision

could be by regulation of amount of protein
present in host (eg by regulated expression)

if use two different cons transposon

can go with addition to
either end + switch
back & forth

SIGNATURE

George Bennett

DISCLOSED TO AND UNDERSTOOD BY

Leslie Sh

DATE

WITNESS

DATE

REDACTED

DATE